



A Brief Analysis of Tissue-Resident NK Cells in Pregnancy and Endometrial Diseases: The Importance of Pharmacologic Modulation

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Abstract: NK cells are lymphocytes involved in the innate and adaptative immune response. These cells are located in peripheral blood and tissues with ample functions, from immune vigilant to tolerogenic reactions. In the endometrium, NK cell populations vary depending on age, hormones, and inflammation. When pregnancy occurs, tissue-resident NK cells and conventional NK cells are recruited to protect the fetus, a tolerogenic response. On the contrary, in the inflamed endometrium, various inflammatory cells down-regulate NK tolerance and impair embryo implantation. Therefore, NK cells' pharmacological modulation is difficult to achieve. Several strategies have been used, from progesterone, lipid emulsions to steroids; the success has not been as expected. However, new therapeutic approaches have been proposed to decrease the endometrial inflammatory burden and increase pregnancy success based on understanding NK cell physiology.

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Citation: Garmendia, J.V.; De Sanctis, J.B. A Brief Analysis of Tissue-Resident NK Cells in Pregnancy and Endometrial Diseases: The Importance of Pharmacologic Modulation. *Immuno* 2021, *1*, 174–193. https://doi.org/ 10.3390/immuno1030011

Academic Editor: Stéphane Terry

Received: 13 May 2021 Accepted: 28 June 2021 Published: 2 July 2021

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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). **Keywords:** NK cells; tolerance; pregnancy; tissue-resident NK cells; conventional NK cells; decidual NK cells; uterine NK cells

1. NK Cell Subpopulations

Natural killer cells (NK) have been considered an essential subtype of lymphocytes involved in innate and adaptive immune responses [1]. In peripheral blood, two main subpopulations have been described; one cytotoxic CD3-CD56dim CD16high, and one cooperative or tolerogenic CD3-CD56bright CD16dim [1]. NK cells can be transformed from one type to another depending on tissue milieu, cytokine or receptor stimulation, or pharmacologic therapy [1,2]. However, NK activity modulation does not come solely by expressing CD16 and CD56 receptor. A complex array of molecules are responsible for NK cell activity. There are activating or cytotoxic receptors, integrin, selectins, killing inhibitory receptors (KIR), PD-1 receptors, CD161, and cytokine receptors responsible for NK cell activity [1,2]. The expression of different receptors may be dependent on the stimulus or tissue milieu.

Reports of NK cells in innate immune response and memory events have led to a better understanding of NK cells' tissue-specific role in the immune response [2–7]. In a similar fashion as tissue macrophages, tissue-resident NK cells (trNK cells) differ in antigen expression and function. Differences have been described for NK cells from skin, liver, adipose tissue, suggesting that trNK cells may be unique depending on the location [6,7]. Most probably, the variance between normal physiological responses, inflammation and remodeling involves trNK cells, macrophages, and the migration of other cell types [6–13]. Chemokines secreted by the tissue may recruit tissue independent NK cells from the blood-stream, and once they arrive at their destination, their cell functions may be modulated by tissue milieu [10–12]. There is still a debate concerning the responses of tissue-resident and conventional NK cells in humans' upon inflammatory stimulus.

In humans, tissue-specific uterine NK cells (uNK) is the most abundant lymphocyte, around 70 %, in decidua and mesometrial tissue (Table 1) [10-13]. These cells highly express

CD56, CD103, CD9, NKp46, KIR inhibitory receptors, CD94 NKG2A, and a low amount of CD16 receptor and killing receptors. As expected, uNK cells are non-cytotoxic and heterogeneous [10–13]. Three different subpopulations have been described in the uterus based on CD56 expression. Two of them express CD117, CD49a, CD103, integrin β 7, CD9, and CD69, and transcription factor Eomes, but one produces IFN γ and the other TGF β /VEGF. The expression of CD103 and CD69 defines the difference in subpopulations (Table 1). A third cell subpopulation expresses CD49a, has an increased amount of Eomes transcription factor, and responds to IL4 [13]. Interestingly, ILC1 cells, associated with Th1 IFN γ transcription and function, are present in the endometrium before puberty. Thus, local ILC1 cells may be involved in the uterine milieu and crucial for the recruitment or maturation of uNK and cNK migration to the tissue in the menarche.

Type of NK Cell	Markers	Cytokine Response
Circulating tolerogenic	CD16 ^{low} CD56 ^{bright} CD94 ^{bright} , NKG2A(C or E)/CD94, CD161.	IL10, TGFβ
Circulating cytotoxic	CD16 ^{bright} CD56 ^{dim} , CD57 ⁺ , CD94 ^{dim} , NKG2D/CD94, NKp30 ⁺ , NKp46 ⁺ , CD62L ^{med}	IFNγ
cNK	CD16 ^{high} CD56 ^{dim} , CD49a ⁻ , DX5 ⁺ , NKG2D/CD94, NKp30 ⁺ , NKp46 ⁺ , CD62L ^{med}	IFNγ
uNK	CD56 ^{bright} , CD16 ^{low} , NKG2A/CD94, CD49a ⁺ , NKp46 ⁺ , integrin β 7 ⁺ , CD117 ⁺ , DX5 ⁻	
uNK1	CD49a ^{high} , CD103 ^{high} , CD69 ⁺	IFNγ
uNK2	CD49a ^{high} , CD103 ^{med} , CD69 ⁺ , KIR ^{high} inhibitory	TGF ^β /VEGF
uNK3	CD56 ^{high} CD49a ⁺ , KIR ^{high} inhibitory, CD69 ^{med}	IL-4
dNK	CD56 ^{high} CD16 ^{low} , CD94 ^{high} , NKp46 ⁺	
dNK 1	CD103 ^{low} , CD9 ⁺ , CD39 ⁺ , CD69 ^{low} , CYP26A1, B4GALNT1 ⁺ , Jag1 ⁺ , TIM3 ^{high} , KIR2DL1 ⁺ , KIR2DL2 ⁺ , KIR2DL3 ⁺ , KIR2DS1 ⁺ , KIR2DS4 ⁺ , LIRB1 ⁺ , NKG2A ⁺ , NKG2C ⁺ , NKG2E ⁺	IFN γ , TGF β
dNK 2	CD103 ^{high} , CD9 ^{low} , CD69 ^{high} , Jag1 ⁺ , CD83 ⁺ , KIR ^{high} inhibitory, ANXA1 ⁺ , ITGB2 ⁺ , TGIT ⁺ , TIM3 ^{low} , NKG2A ⁺ , NKG2C ⁺ , NKG2E ⁺	TGFβ/VEGF
dNK 3	CD103 ^{low} , KIR ^{high} inhibitory, ITGβ7+, CD74 ⁺ , CD160 ⁺ , KLRB1 ⁺ , ITGB2 ⁺ , TGIT+	TGFβ/IL-4

Table 1. General Characteristics of NK cells.

The table represents the described markers for different cell populations and the responses to cytokines. The term cNK are conventional NK cells, uNK are uterine resident NK cells, dNK are decidual NK cells. The acronym LIRB1 corresponds to Leukocyte immunoglobulin-like receptor-1, B4GALNT1refers to β 1,4 N acetylgalactosamine transferase 1, CYP26A1 corresponds to Cytochrome P450 26A1, ANXA corresponds to annexin A1, ITGB2 corresponds to integrin subunit beta 2, TGIT corresponds to T cell immunoglobulin and ITIM domain.

In mouse, a new classification was postulated of innate lymphocytes (ILC) as ILC1, ILC2, ILC3, NK tissue-resident (trNK), and conventional NK cells (cNK) [5–7]. Recent reports have shown that trNK cells express CD49a, but lack the expression of DX5, and the reverse is true for cNK [8]. Also, cNK cells are more cytotoxic than trNK and ILC counterparts [5,8–12]. Based on this definition of innate lymphocytes, Sojka and coworkers [12,13] named the NK cells in the uterine tissue as trNK, not uNK cells. Their remark is based on the fact that, in pregnancy, cNK migrate to the tissue and cooperate with trNK cells. The two NK cell subpopulations, tissue-resident and conventional, are distinguishable [10,12,13]. Nfil3 and Eomes are the required transcription factors for cNK cells and not for uterine trNK cells [12]. When pregnancy occurs, trNK and cNK cells increase in the decidua, providing the tolerogenic model for a normal pregnancy [12,13]. The trNK are closer to the trophoblasts, and the cNK cells are in the periphery. Once labor starts, the cNK presence decreases in the endometrium, while the number of uterine trNK cells diminishes in the puerperium [12]. In this particular physiological event, the cooperation among different cell populations and the tissue involved is crucial for fetal survival. Nonetheless,

the tissue events in which NK cells are involved are not necessarily dependent on specific subpopulations circulating NK cells in the peripheral blood [6,8,10–13].

2. NK Cells in Pregnancy

Progesterone is essential in reproduction and pregnancy maintenance. The hormone induces the transcription and secretion of progesterone-induced blocking factor (PIBF) [14,15]. In turn, PIBF levels are increased in normal pregnancy and very low in recurrent pregnancy failure [16]. Moreover, the progesterone stimulates protein glycodelin A (GdA), or human placental protein-14 binds to NK cells CD16 low CD56bright NK cells purified from peripheral blood and transforms them into dNK-like cells [17,18]. Sialylated glycans expressed on glycodelin A are critical for binding to the receptor [17]. In addition, Glycodelin A stimulated cells to control endothelial cell angiogenesis by secreting vascular endothelial growth factor (VEGF) and trophoblast invasion by secreting insulin-like growth factor-binding protein 1 (IGFBP-1) [17].

Studies in pregnancy loss and preeclampsia have raised important questions concerning the role of NK cells in embryo implantation [9–19]. Fetal expression of MHC-I and its recognition by KIR receptors on NK cells, along with NKG2A/CD94, are critical for dNK cells (Table 1). On the contrary, the role of cNK cells is not tolerogenic; it protects the decidua against pathogens and abnormal cells. The cytotoxic response of cNK cells depends on NKG2D/CD94 and KIR activating receptors, NKp30, NKp46, and not by NKG2A/CD94 and KIR inhibitory receptors and their ligands. Activation of cNK cells may lead to a robust cytotoxic response against the embryo leading to pregnancy termination [11,19–22]. However, cNK cells are also critical for vascular remodeling, an important event to maintain blood flow to the fetus, often impaired in preeclampsia. The tolerogenic response of dNK cells is dependent on TGF β levels, while IFN γ activates cNK cells. Thus, a balance between TGF β production inducing the tolerogenic response of NK cells or IFN γ inducing inflammatory responses is critical to determine pregnancy outcome [11,19–22].

Huhn and coworkers [23] were able to determine, in healthy human pregnancy, different cell populations in the decidua (Table 1). They identified three decidual NK cell subpopulations (d1, d2, and d3), ILC3, and a group of proliferating NK cells using mass cytometry [23]. When dNK cells were stimulated with PMA and ionomycin, dNK2 and dNK3 secreted more chemokines than dNK1. The secretion included the chemokine C motif ligand (XCL1), a cytokine able to activate maternal dendritic cells and fetal extravillous trophoblasts. The d2 and d3 NK cells express a high amount of KIR inhibitory receptors [23]. Nevertheless, KIR antigen expression in dNK1 cells correlates with granzyme B granule content in the cells suggesting that these cells are more prone to be cytotoxic. These results indicate that KIR receptors control fetal development and could be involved in eliminating abnormal trophoblasts [24]. These issues have also been discussed in a recent review [19]. In summary, uNK cells are similar to dNK cells in the expression of several antigens; however, some others are only present in dNK cells (Table 1).

The involvement of different dNK cell subpopulations in embryo implantation and maintaining pregnancy still requires more research. Guo et al. [24], analyzing single-cell NK by qPCR, reported that a subset of dNK cells, previously defined as a protective NK cell for embryo growth, is diminished in patients with spontaneous abortion. Even though the decrease of this subpopulation may be due to different events, their assessment may be critical for determining therapeutic success.

A shift in the control of tissue subpopulations can be observed in different stages of normal pregnancy. In the first trimester, NK cells are prone to protect the fetus from pathogens; however, this protection decreased in term pregnancies [19,22,23]. In term pregnancies, dNK cells, usually tolerogenic, have a higher cytotoxic response against K562 than those of the first trimester [22,23]. This effect may be due to an impaired inhibitory response due to a downregulated expression of inhibitory receptors recognizing HLA-C antigens or HLA E and G similarly as cNK cells [22,23]. Also, the dNK cells from the first trimester differ from that dNK obtained in term pregnancies based on proteomic data.

It is unclear if the cNK cells redistribute to other tissue or circulation after pregnancy termination and if the dNK cells transform into uNK cells to aid endometrial tissue repair after pregnancy.

The role of cNK cells on pathogen has become more evident in the latest years. cNK cells have been involved in protecting the fetus against infections like Toxoplasma gondii [25,26]. During Toxoplasma infection, Tim-3 is involved in dNK impairment, and consequently, cNK may be involved in pregnancy termination by NK cells [25,26]. The response is dependent upon the production of IFN γ and the downregulation of KIR inhibitory receptors.

3. NK Cells and Endometrial Disease

A first approximation of the importance of the role of hormones in endometriosis was by Loverro and coworkers [27]. Mizumoto [28] and Eriksson et al. [29] contrasted previous reports demonstrating the presence of suppressive leukocytes in the endometrium, and Eriksson et al. [29] defined the phenotype of uNK cells and their response to TGF β . One essential difference between NK cells from peripheral blood and uNK cells is the response to hypoxia [30–32]. Furthermore, the peripheral NK cells and uNK cells respond differently to TGF β when the cells are incubated in hypoxic conditions ex vivo [31,32]. These results suggest a significant difference in hypoxia-inducing transcription factor 1 and, consequently, substantial changes in physiological responses.

In mouse endometriosis models, trNK cells express higher amounts of inhibitory KIR receptors [33]. This increased expression does not parallel with an increase in HLA E and HLA G molecules, physiological ligands of such receptors, suggesting that the inflammatory response is not physiologically controlled. Other MHC class I receptors and NKp46, CD103 (integrin alpha E), seem to be related to NK cell attraction and increased cytotoxic responses by cNK cells [33,34]. In aged mouse and humans, the resolution of endometriosis is delayed [34]. Chronic inflammation in endometrial tissue decreases the telomerase activity of several cells, impeding their optimal physiological response in mouse [34] and humans [21,35–39]. trNK cells could also be affected by the low telomerase response [34]. On the other hand, senescent cells may activate the cytotoxic response of cNK cells [34–39] against autologous cells and, in consequence, generate more cell death maintaining the local chronic inflammatory response.

In humans, NK cells, macrophages, and T cells are responsible for maintaining the local inflammatory response [35–40]. The role of TH17 and IFN γ has been widely discussed; however, the control of inflammation in the tissue is not achieved in many patients despite surgery or other therapeutic options raising the question of a wider variety of endometrial diseases [21,36–39]. One of the often-overlooked events is autoantibodies, which can play an essential role in maintaining the inflammatory response [40–42].

The frequency of endometrial cancer is high in developed countries as compared to other cancers [43]. The occurrence of this type of cancer has been associated with ageing and obesity [43]. Despite the solid statistical association between ageing and obesity to tumour incidence, little is known about the immune response in this type of tumour except for T CD8 cytotoxic cells [44]. The generation of a tumour in the endometrium associated with age and obesity has raised important questions concerning the pool of circulating and tissue-dependent NK cells [43-46]. The trNK cells do not represent a cell type able to induce a proper immune vigilant response [45,46]. In an analysis of menstrual blood, NK cells were shown to express CD103 [47], an antigen expressed in T regulatory cells [48]. Two populations were described based upon the CD103 marker [47,48]. The CD103 positive cells express inhibitory KIR, Tigit, and TIM3 proteins and inhibit local immune response [49,50]. The CD103 negative counterparts have low expression of those markers and high cytotoxic markers; they are cytotoxic against the tumour [49,50]. The number of inhibitory receptors and suppressive cells increases depending on the severity of the disease [49,50]. The production and secretion of chemokines and IL-1 β and IL-6 by these cells are decreased, suggesting reduced cell recruitment to the tumour [49,50].

Several reports on the importance of KIR receptors in endometriosis [51,52] and the expression of NKG2D [53]. However, more analysis is required because of the contradictory reports on uNK cells in endometriosis and inflammatory events related to pregnancy loss [54,55].

4. NK Cells in Autoimmune Diseases

During the screening of possible causes of infertility or recurrent pregnancy loss, the presence of autoantibodies may be relevant. The presence of autoantibodies may be independent of peripheral lymphocyte or NK cell populations, but may be dependent on tissue subclinical inflammatory responses.

Women with autoimmune diseases with the proper therapeutic conditions may undergo pregnancy without major complications. The most common autoimmune disease is anti-thyroid antibodies [41,42,56–58]; however, the most difficult to treat is antiphospholipid antibody and lupus. The main issue depends upon the control of autoantibodies and thrombosis. Most patients with subtle inflammatory reactions may benefit from progesterone or anti-inflammatory therapy [40].

Autoantibodies may be responsible for NK cell activation in the decidua; however, the mechanism is unclear [41,42,56–58]. Control of thyroid hormone can be achieved pharmacologically. Vitamin D appears to have a protective role in this process as human chorionic gonadotrophin [56–58]. Both induce a tolerogenic immune response [56–59]. However, anti-nuclear and antiphospholipid antibodies may require special medical treatment. Zhang and coworkers [41] were able to show a correlation of NKG2D expression with antiphospholipid antibodies in obstetric patients. These results support the hypothesis of downregulation of inhibitory KIR receptor expression in patients with autoimmune diseases. It would be interesting to study the possible role of small HLA molecules in modulating NK cell responses in these patients.

Jiang and coworkers showed the importance of NETosis and dNK cells in lupus pregnancies [60]. The critical issue seems to be related to the inflammatory burden based on cell death at the endometria [60]. In addition, NETs may induce dNK modulation depending on DAMP receptor signaling, which would downregulate KIR inhibitory receptors, enhancing KIR activating receptor [60]. Probably, impaired production of HLA E and G, ligands of inhibitory receptors, may also play a vital role in dNK activation.

5. NK Cells in Obesity

In adipose tissue, trNK cells seem to be important not only in maintaining adipose tissue control, but they may be involved in the subtle inflammatory activation that may lead to insulin resistance and metabolic syndrome [46,61–63]. In the breast mouse tumour model, adipose tissue increase and high-fat diet are related to more tumour growth, suggesting a skewed response of NK cells against the tumour [46,61–63]. In particular, Spielman and coworkers [63] injected mammary tumour 4T1-luc2 cells in the mammary adipose tissue of BALBc animals in which ovaries were surgically removed and showed that NK cells close to the tumour had a higher expression of NKG2D despite a decreased expression of NKp46 and an increased amount of adipose tissue. These results propose that there is a link between adipokines and circulating and trNK cells [64]. Even though leptin and adiponectin do not alter peripheral blood NK cells cytotoxic response in vitro, there is evidence of the increased incidence of endometrial, ovary, and mammary tissues cancer in obese humans and animals [46,65].

Another alternative that has not been explored in detail is single or multiple genetic polymorphisms of critical proteins, receptors, or signal transduction pathways that may be relevant for NK cell modulation and tissue migration [46]. Studies with the NK cell line NK-92 suggest that several exciting analyses could be performed using migration and invasion [66–69]. For example, STAT 3 was involved in Tim-3 dysregulation of dNK, which involves immune response against pathogen and tissue rejection [27,67–69]. The migration of tissue-resident NK cells from obese individuals has not been studied thoroughly in

different metabolic disorders related to circulating hormone levels and could be relevant to understand the possible effect on uterine or decidual NK cells.

Pregravid and gravid obesity seem to alter the immune cells involved in the endometrium [70–72] and predispose women to gestational diabetes. Maternal obesity also alters uNK activity [70–72]. Even though in NK cells of gravid obese women, the expression of KIR2DS1 is increased over KIR2DL1, there is no increase in cytotoxic response. Yet, the interaction of the activating receptor HLAC2 induces the secretion of TNF α , which affects the local inflammatory milieu and may affect fetal development and may predispose to preeclampsia [70–72]. It is unclear whether nutritional restriction or diet modulation may affect uNK cells in obese pregnant women. Conversely, the effects may be assumed to be similar to those observed in mammary tumour-challenged mice [73].

6. NK Cells in Infectious Diseases in Pregnancy

Control of infectious diseases is essential during pregnancy since they can be lifethreatening for the embryo and fetus [74–76]. The most dangerous pathogens in pregnancy are influenza, SARS CoV-2, malaria, toxoplasma, hepatitis, Zika virus, herpes simplex virus, human cytomegalovirus, Ebola, dengue, measles, and smallpox [74–76]. In addition, pregnant women may be more susceptible to pathogen infection, malaria infection, HIV infection, and listeriosis. The susceptibility may be due to the tolerogenic environment generated during pregnancy. For example, in SARS CoV2 infection, there seems to be an increased activation of NK and T cell in the maternal-fetal interface, despite the absence of virus in the tissue [77]. This effect may be due to inflammatory cytokines generated upon viral infection.

In Listeria monocytogenes, the role of uterine NK cells seems to protect from the infection, although it is not crucial for its response as observed in the mouse model [78]. However, other pathogens like mycobacteria have been barely studied. There has been a proposal for a BCG vaccine for endometriosis since it is known that BCG activates NK and NKT cells [79]; however, there have not been well-conducted trials to test that hypothesis.

One of the critical elements for the elimination of pathogens by NK cells is the presence of KIR. In an exciting analysis performed by Omosun and coworkers [80,81], KIR receptors' role in placental malaria and perinatal transmission of HIV-1 was analyzed. Inhibitory KIR2DL2 and KIR2DL3, alleles of the same locus, negatively affect malaria and malaria/HIV co-infection. Furthermore, the maternal KIR genes KIR2DL2, KIR2DL5, KIR2DS5, and KIR2DS2, were associated with decreased HIV-1 transmission from mother to child [80,81].

KIR expression is crucial in protecting the fetus and may play a pivotal role also with preeclampsia associated with the inflammatory response generated by the pathogen [82–86]. An association between KIR/HLA class I mismatch and preeclampsia suggest that there may be more than one mechanism involved between NK cell activation upon pathogen-induced local inflammatory response and preeclampsia [85,86]. Future analysis should be directed towards understanding the phenomenon, and probably using small HLA inhibitory molecules HLA E/G could decrease the inflammatory burden. For example, soluble HLA-G induces apoptosis of dNK cells after Toxoplasma gondii infection [87].

Innate immune antiviral response plays a crucial role in the response. In cytomegalovirus infection, Tomac and coworkers [88] were able to show that low viral load and high viral load affect the corpus luteum differently in a murine model. There is local inflammatory activation at low viral load, but pregnancy continues; there is a massive immune response at high viral load, leading to pregnancy loss. Moreover, in Zika virus infection, there is evidence of memory-like NK cells, and the expression of CD27 characterizes it [89]. However, these experiments, carried in the mouse model of the disease, may not be parallel to the infection in humans since the viral escape, in humans, seems to be dependent upon upregulation of MHC class I [90]. Herpes simplex virus may induce embryo damage by decreasing the expression of HLA G, critical in local tolerogenic responses [91].

In patients with hepatitis B and recurrent pregnancy loss, the virus induces a decrease in NK cell function in peripheral cells [92]. A similar impairment in NK cell cytotoxicity was observed in hepatitis C virus-infected patients [93]. Furthermore, diminished NK responses are also observed in dengue viral infection and may play a role in the disease [94]. Thus, dysregulation of NK cells in these viral infections may be significant for peripheral NK cells and trNK cells.

NK cells are activated and can provide protecting memory responses in Ebola viral infections [95]. This protective response seems to be essential for liver NK cells, a target tissue. Thus, the protection against the virus may be critical also in uterine NK cells and decrease cell mortality.

Only a few reports have described the local immune response against measles and smallpox in pregnancy.

The differences in NK cell response to viral infection observed in the murine model cannot be assumed to occur in humans, reviewed recently [96,97]. In particular, several assumptions based upon the response of trNK cells may not be extrapolated since ILC cells play an essential role in mice and a less prevalent role in humans.

7. Pharmacological Modulation of NK Cells

In general, normal pregnancy requires a tolerogenic response that is controlled at the tissue level. Pharmacologic modulation of this effect is essential in order to achieve that goal [98]. The therapy has been primarily based upon indirect knowledge from animal models and in the analysis performed in patients with recurrent abortions due to non-genetic or pathogenic events [98–100]. However, different medical conditions in which therapy may substantially affect pregnancy outcomes may be related to NK function.

One crucial analysis is based on the fact that peripheral blood NK cells may not be appropriate to determine treatment effect [98–100]. Variations, upon treatment, on circulating cell quantities, and a decrease in cytotoxic responses may be due either to changes in the activation markers or changes in subpopulations based upon cell maturation. Due to the variety of receptors and signals involved in NK physiology, it is challenging to determine which specific antigen or receptor could be a critical biomarker to ascertain treatment responses.

NK cells in recurrent spontaneous abortion have been several pharmacological therapies used [98–100]. Some therapies are based on a general anti-inflammatory rationale, screening peripheral blood NK cells subpopulations and cytotoxic activity [98–100]. However, treatment efficiency depends on pregnancy success and complete pregnancy with alive newborns [99,100]. In some cases, therapeutic modulation has been shown to increase the number of genetic malformations and the possibility of preeclampsia, eclampsia, or HELLP syndrome [98–100]. Hence, the modulation of tissue-resident NK cells and conventional NK cells may alter the local biological response.

Table 2 summarizes different therapies involved in NK cells and their involvement in pregnancy.

7.1. Progesterone

Progesterone is an essential hormone that may be given orally or applied topically for pregnancy maintenance [101–103]. NK cells do not have receptors for this hormone; however, T cells do [102–104]. The factor induced by progesterone, PIBF, generates a tolerogenic TH2 response in the endometrium and consequently may affect NK cell migration and response [105]. Miko et al. [106] reported an inverse correlation between PIBF levels and local leptin levels and leptin receptors involved in local progesterone production and trophoblast growth. These results parallel that described blocking PIBF in an experimental mouse model. PIBF inhibition significantly increased miscarriages despite successful implantation. The increase in miscarriage was associated with an increase in NK activity [104–106]. In the equine model, an exciting interaction between galectin and PIBF has been observed protecting pregnancy [107]. Thus, the effect of progesterone is dependent upon glycodelin A and PIBF transcription and secretion. They, in turn, provide the critical tolerogenic milieu for embryo implantation and pregnancy.

In the presence of progesterone, stromal and dendritic cells secrete IL-15 and IL-18 critical for NK cells [105,108]. A significant increase in IL-15 level is observed in the secretory phase compared to the proliferative phase of the hormonal cycle and then during early pregnancy in parallel to progesterone levels [105,108]. A negative correlation was also found between IL-18 and the number of uNK [109]. Murata H and coworkers [110] recently reported the importance of the heart- and neural crest derivatives-expressed transcript 2 (HAND2) and inductor of IL-15 secretion by human endometrial stromal cells induced by synthetic progesterone. Then, it is assumed that this IL-15 produced is critical in the role of NK in the decidua.

In an interesting study involving in vitro fertilization, it was found that serum progesterone levels are critical for pregnancy success and termination, suggesting that modulation of decidual cells by progesterone is crucial [111]. Furthermore, progesterone is required to induce a tolerogenic milieu in the endometrium and the decidua.

The hormone is generally used as an immunosuppressor. There are therapeutic guidelines to use in endometriosis, and it has been used to prevent abortion and preterm delivery [112]. In general, there are three pharmacological approaches: oral formulation, injections, and micronized hormone. It was hypothesized that progesterone absorption through the vagina may downregulate the endometrium's inflammatory milieu [112]. However, there are differences in pharmacodynamics depending on the hormone preparation. The use of progesterone regulates glucose transport, enhancing endometrial receptivity to the embryo [101].

Progesterone's effect on different cell types and trophoblast, like the newly described variety of trophoblast expressing HLA-G, is unknown [113]. However, in this case, the critical element modulates inhibitory signals through the KIR receptors, which can be crucial for embryo implantation and successful pregnancy.

7.2. Low Molecular Weight Heparin

Experiments ex vivo performed many years ago highlighted the role of heparin decreasing cytotoxic response [114]. Heparin was also shown to increase fetus stability, diminishing pregnancy loss. The effect of heparin is attributed to modulation of endothelial cell responses, relaxing the vasculature, decreasing resistance, and hence protecting the patient from preeclampsia [114–116]. However, ex vivo experiments using macrophages and helper T cells stimulated with heparin were more inflammatory than tolerogenic [117]. This unanticipated result may be due to the lack of other cells that contribute to the cell response. A recent report reveals that the effect of heparin as the antitumor drug does not involve NK cell activation, suggesting that heparin instead activates Th1 and hence NK cells are more cytotoxic against the tumour [118]. More research is required to ascertain the importance of this drug on in vivo NK cell modulation in pregnancy.

Patients with antiphospholipid antibodies and with thrombotic pathologies are treated with heparin during pregnancy. There are several hypotheses on the possible mechanisms of heparin; however, none of them involved NK cell response [118,119]. Thus, heparin's role in preventing thrombotic events may be crucial for the embryo or fetal survival. The use of heparin as an immunomodulator is complicated to envision; however, the effect of heparin binding to receptors on different cell types and modulating cellular processes may, in turn, affect NK cell response [118,119]. Therefore, more research should be performed to ascertain the possible effect of this and other glycosaminoglycans on NK cell function.

7.3. Corticosteroids

Corticosteroids have been found to modulate the NK cell response [120]. Prednisolone treatment of NK in vitro suppressed NK cells cytolytic activity against K562 cells [121]. This suppressive effect of steroids was observed on the cytotoxic CD16+ NK cells [121]. Before pregnancy, the effect of corticosteroids has been related to a decrease in the subclinical

pro-inflammatory burden [122,123], and it may favor embryo implantation. However, it is unclear what corticosteroids may affect local trNK cells or the migration of cNK cells.

A recent review by Li and coworkers [124] revised the PD1/PD1 L axis and pregnancy role. When there is an increase in the expression of PD1 in endometriosis [125], there is less probability of pregnancy maintenance. In cancer therapy, the difference in response is clear using dexamethasone or prednisone involving PD1/PDL1 L axis [126]. Therefore, this axis should be studied in more extensive trials. On the other hand, if steroids down modulate PD1 and decrease the cytotoxic or pro-inflammatory responses of stimulated NK cells, then embryo anidation and pregnancy will be successful.

7.4. Intravenous Immunoglobulins (IVIg)

The pharmacological mechanisms of IVIg enhancing embryo implantation and successful pregnancy have not been studied thoroughly. In peripheral blood, it was reported: a decrease in NK cytotoxic activity, a decreased amount of activated T and B cells, an increase in tolerogenic T cells, along with a reduction of activated antigen-presenting cells [127]. A decrease of autologous antigens upon treatment is suspected [127–129]. In a meta-analysis of 13 trials, Christiansen et al. [129] were unable to determine the impact of IVIg on live births. However, if the treatment started before conception, the results were better. Similar outcomes were encountered by Abdolmohammadi-Vahid et al. [130] in four clinical trials. More well designed clinical trials, including monitoring NK cell cytotoxic and tolerogenic responses, are required.

Two exciting issues have not been well described in this treatment. First, it is believed that intravenous immunoglobulins would decrease the number of antibodies against their own ligands and decrease autoimmune responses [127–131]. Yet, it is unclear if IVIg may enhance antibody-dependent cellular cytotoxicity on NK cells. Second, ADCC may increase the elimination of unwanted cells in different tissues and consequently decrease the inflammatory burden detrimental to embryo implantation and pregnancy maintenance.

7.5. Lipid Infusions

Lipid infusions, particularly Intralipid, have been suggested as alternative therapy for recurrent abortions since these lipids seem to modulate NK cell function and promote trophoblast invasiveness [132,133]. In mouse models and ex vivo studies, intralipid suppressed abnormal NK cytotoxic [132–135]. However, the results of the meta-analysis have not been conclusive. On the other hand, omega-3 fatty acid infusions decreased inflammatory burden (TNF- α , IL-1, IL-6 or IL-8 [133–136]. However, again, the lack of adequately designed trials has limited this therapy in recurrent abortions.

Our group reported an increase in NK proliferative and cytotoxic responses when human NK cells were stimulated in vitro with chylomicrons [137]. In addition, the supplementation with fatty acids modulated the expression of different receptors on the NK membrane [138]. Probably, the use of intravenous intralipid would modulate NK subpopulations and consequently favor NK maturation and migration.

7.6. Vitamin D

Several years ago, Evans and coworkers [139] reported that decidual cells could synthesize 1,25-dihydroxy vitamin D3 and that this production is higher in the first trimester of pregnancy. These results raised essential questions on local cell tolerance and the importance of vitamin D in tolerogenic responses. Tamblyn and coworkers [140] showed significant differences in vitamin D3 between uNK and peripheral blood NK cells, suggesting different overall signal transduction responses. Even though the authors concluded that uNK cells and peripheral blood NK cells are not the direct targets of vitamin D, the difference in cell responses suggests that the uptake of vitamin D3 can be an accessory signal for local uNK tolerogenic responses.

Vitamin D3 was shown to modulate NK cytotoxic response [141], an event that has been linked to an increased success rate of pregnancy [142]. Ota and coworker [143] per-

formed a gene polymorphism of methylenetetrahydrofolate reductase C677T in patients with recurrent abortion [144]. Patients with the TT polymorphism had higher homocysteinemia levels, lower vitamin D3 levels, nevertheless, higher NK cell cytotoxic responses against K562 cells [144]. These results suggest a relationship between folate metabolism with vitamin D3 levels and NK cytotoxic responses. Supplementation of vitamin D3 and methyl folate in these patients will probably increase pregnancy success.

In a recent review on vitamin D and pregnancy, Zhao et al. [144] discussed the importance of this vitamin on pregnancy loss associated with antiphospholipid syndrome and modulation of Th1/Th17 axis in recurrent pregnancy loss. It is suggested that the effect of vitamin D disrupting this pro-inflammatory Th1/Th17 axis affect NK responses and hence pregnancy maintenance. More research has to be done to verify this hypothesis.

7.7. Exosomes

Exosomes are extracellular vesicles that originate from the endosomal compartment of cells. The structures contain protein, DNA, RNA, and/or miRNA of the cells that secrete them. Exosomes have been used as biomarkers in different reproductive diseases [145–151].

Wu and coworkers [149] analyzed the miRNA in samples from patients with varying types of endometriosis to understand the regulation of different pathways. They then were able to explore the most relevant miRNA in endometriosis [148–150]. This information is critical since directed therapies depending on endometriosis may provide new elements to control the inflammatory disease and aid in fertilization [148–166]. In addition, exosomes contain miRNA and other proteins. These miRNAs decrease the inflammatory burden by blocking the transcription of inflammatory cytokines and, consequently, induce tissue repair crucial for embryo annidation and pregnancy survival [149–151].

The use of endosomes for therapy has raised the interest of several groups in recent years [145–149]. In addition, the experience with other disease has provided new tools for generating individual and targeted analysis to decrease the inflammatory response and provide the critical tissue milieu for implantation and pregnancy [145–151].

7.8. Other Therapies

A recent review by Busnelli et al. [104] revisited several procedures regarding recurrent pregnancy loss. The intrauterine infusion of autologous peripheral blood mononuclear cells (PBMC) seems to reduce endometriosis [105]. Also, platelets infusion can increase local progesterone production, probably by releasing TGF β [152]. Thus, the infused cells provide a tolerogenic milieu for embryo implantation. Also, a mild positive effect of subcutaneous G-CSF injection inducing clinical pregnancy post embryo implantation was observed [104]. One possible mechanism of tolerance induced by G-CSF is the inhibition of NK cytotoxicity activity [104]. It is nevertheless unclear why the beneficial effect of G-CSF is subcutaneous and not intrauterine.

Other therapies involve platelet-rich plasma in the uterine cavity and the use of different stem cells [149–155]. The goal of the platelet-rich plasma is related to local TGF β release and consequently promote NK differentiation [149–155]. There have not been conclusive studies using this therapy and other therapies involving various stem cells and allostimulation [155]. There is still room for more directed treatments, which would increase the recruitment and modulation of NK cells. However, it is essential to recognize that a balance of NK subpopulations, cNK, uNK, and dNK, is required for normal physiological responses. The immune vigilant role of NK cells is also crucial for eliminating pathogens and abnormal cells, and non-viable embryos.

Modulation of PD1/PDL1 is also essential in understanding NK responses in the endometrium and the possible role of this diad in preeclampsia [156–160]. This diad's mechanism may depend upon the inflammatory burden and the expression of markers of downregulation on NK cells. The inflammatory response generated by pathogens of tumour cells in the endometrium involves NfkB, which also seems vital in uterine artery remodeling [160].

Treatment like medroxyprogesterone acetate was shown to increase NK cell activity in menopause [161]. This modulation may also be relevant in other conditions like polycystic ovary syndrome, in which hormonal dysregulation may affect endometrial NK cell responses [158]. Waiyaput and coworkers [162] reported that treatment with combined contraceptive pills increased the migration of NK cells, suppressive macrophages, and tolerogenic T cells to the inflamed endometrium of menopause women. In addition, protopanaxadiol, an antioxidant ginsenoside compound, was reported to induce the expression of tolerogenic antigens on NK cells [163]. Thus, hormone therapy radical scavenging causes a protective response in the endometrium.

Interestingly, new therapeutic uses essentially for antitumor responses as LAIR-2 (LAIR-1 inhibitor) and P4H shRNA (collagen inhibitor) modulate NK cytotoxic responses and may provide exciting options in the future for peripheral and trNK cells [164]. JAK kinase inhibitors were shown to modulate peripheral NK and ILC1 cells with stimulating effects on immune response regulation [165]. New studies may focus on the use of therapy usually used for treating tumors. Aromatase inhibitors may also be relevant for combined therapies in endometriosis.

The immunomodulatory effect of Mesenchymal Stem Cells (MSCs) on NK cells has been a matter of discussion for several years [145,146]. In a mouse model of recurrent abortion, when MSC cells were co-cultured with primed, inflammatory NK cells, MSC induced a shift in cytokine production and tolerogenic responses [145]. This result suggests that MSC cells may influence a tolerogenic microenvironment in early pregnancy. This effect has to be assessed in clinical studies. It is unclear if another type of stem cell could also be involved in the treatment.

Another promising target for therapy is G-CSF since the cytokine is modulated in the endometrium and may facilitate the endometrial repair and NK cell migration. Studies of the role of this cytokine in pregnancy and endometrial cancer are underway.

It can be concluded that the different therapeutic schemes should be concentrated on the signal transduction responses of NK cells. Directed therapies would enhance the expression and functions of tolerogenic molecules rather than activating molecules, promoting embryo rejection and pregnancy termination. Figure 1 illustrates the crucial pathways that should be modulated with new types of directed therapy. The role of cytokines or other soluble factors may not be as effective as other factors, considering the difference in peripheral NK cells compared with tissue NK cells. In addition, the reduction of the inflammatory milieu has to be achieved, most probably with complementary therapy. The effect of cytokines and fatty acids may depend on the NK cell type and the type of fatty acid involved. Future research should be directed to study the mechanism of NK modulation by using small molecules that would control specific signal transduction pathways. Several therapies employed nowadays still require proper validation.



Figure 1. This figure summarizes the general aspects discussed in the review. Some of the most relevant receptors in human NK cells are represented along with the mechanism of the different therapies reviewed. The inhibitory effects are depicted in red, and the activating effects in blue. The receptors described in yellow represent an effect that can induce activation or inhibition. In the case of fatty acids, the effect encountered depends on the type of fatty acid, and in the case of the cytokines, G-CSF and IL-15, it depends on the NK cell type.

8. Conclusions

There are several populations of NK cells that may play an essential role in immune response in pregnancy. The presence of different subpopulations of NK cells in tissues and its possible migration, dependent on hormonal stimulus, has raised important questions concerning pregnancy's physiological and pathological responses. However, screening the peripheral blood NK cells number and cytotoxic function provides only partial information concerning the tissue-specific effect. More research should be performed to identify potential markers of therapy response that could be reliable and easily detectable.

Pathogens can activate local NK cell responses and can jeopardize the pregnancy. Different viral, parasitic, and bacterial infections affect local NK cells, essential for the embryo or fetal survival. Most of the responses can be modulated by KIR receptors, KIR ligands, and small tolerogenic small HLA molecules. However, most of the studies have been carried out either in the animal model with few cases in human counterparts, which have to be analyzed carefully. More research should be focused on exploring the effect of pathogens and therapy. In the current pandemic, new concepts of viral response, clearance and use of vaccines have been achieved in the emergency. Some of this knowledge can be extrapolated in other infectious.

Local hormones and other therapies seem to provide essential insights into pregnancy outcome rather than lipid emulsions. Steroids are vital compounds for decreasing the inflammatory burden and consequently modulate NK cell activity. However, new formulations of steroids should be designed to enhance treatment efficiency and reduce unwanted responses. Based on the analysis of endometrial biopsies, genetic and molecular analysis, and NK cell populations, the categorization of patients may enhance treatment responses.

Further research should be performed to increase therapy efficiency. Exosomes and mesenchymal stem cells could be essential for individualized therapy. Other strategies may soon be available and will provide more insights into the role of NK in pregnancy outcome.

Effect on Peripheral NK Local Cells Animal Model References **Treatment Type** Cells (Humans) The indirect effect Inhibition on NK through T lymphocyte Increase tolerogenic cytotoxicity by the and stromal cell-cell response. There is an Progesterone [103–113] progesterone-induced cytokine secretion indirect effect of T cells Progesterone-induced blocking factor and stromal cells. blocking factor The indirect effect Low molecular weight heparin No direct effect No direct reports [114–119] through T cell? Decrease in cell number Downregulation of Increase in Corticosteroids and decrease in [120–126] tolerogenic responses PD1/PD1L expression cytotoxic response. Decrease in TH1 Reduction of deficient autoimmune cells. IVIg Not reported [127–131] stromal cells. Modulation of NK cells Decrease of the Reduction of local inflammatory burden transcription and Suppression Lipid infusion [132-138] secretion of IL15 cytotoxicity in vitro and reduction in NK cytotoxic activity and IL18 Modulation of iNKT Decrease of in vitro Increase in Vitamin D cells which may affect [139–144] tolerogenic responses cytotoxic response NK cells Increase in cytotoxic Decrease inflammatory Increase tolerogenic NK Exosomes response against burden [145–151] cell responses tumour cells increase tolerance Increase tolerogenic Increase in local Intrauterine PBMC Platelets [152-155] response via TGF^β tolerogenic response Increase in NK Medroxyprogesterone acetate Local decrease of IL-15 Not described [161,162] cell activity Decrease peripheral NK Not described Other small molecule therapy Regulation of dNK [164,165] cytotoxic response Increase in There is an indirect effect Increase in G-CSF tolerogenic response [166]on NK cytotoxic response tolerogenic response Tim-3 dependent

Table 2. Pharmacologic modulation of NK cells. The table represents the significant finding with the different described treatment for modulation of NK cell populations and functions.

Author Contributions: Conceptualization; investigation; writing J.V.G. And J.B.D.S.; funding acquisition, J.B.D.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by a grant from the Ministry of Education, Youth and Sport, Czech Republic: Molecular and Cellular Clinical Approach to Healthy Ageing, ENOCH (European Regional Development Fund project No. CZ.02.1.01/0.0/0.0/16_019/0000868, IMTM #869/V19).

Acknowledgments: The authors would like to thank Isaac Blanca and Ivana Oborná for helpful discussions.

Conflicts of Interest: The authors declare no conflict of interest.

References

- 1. Chen, Y.; Lu, D.; Churov, A.; Fu, R. Research Progress on NK Cell Receptors and Their Signaling Pathways. *Mediat. Inflamm.* 2020, 2020, 6437057. [CrossRef] [PubMed]
- 2. Goodier, M.R.; Riley, E.M. Regulation of the human NK cell compartment by pathogens and vaccines. *Clin. Transl. Immunol.* **2021**, 10, e1244. [CrossRef]
- 3. Beaulieu, A.M. Memory responses by natural killer cells. J. Leukoc. Biol. 2018, 104, 1087–1096. [CrossRef]

- Beaulieu, A.M. Transcriptional and epigenetic regulation of memory NK cell responses. *Immunol. Rev.* 2021, 300, 125–133. [CrossRef]
- 5. Huntington, N.D.; Cursons, J.; Rautela, J. The cancer-natural killer cell immunity cycle. *Nat. Rev. Cancer* 2020, 20, 437–454. [CrossRef]
- 6. Sojka, D.K. Uterine Natural Killer Cell Heterogeneity: Lessons From Mouse Models. Front. Immunol. 2020, 11, 290. [CrossRef]
- 7. Zhou, J.; Tian, Z.; Peng, H. Tissue-resident NK cells and other innate lymphoid cells. *Adv. Immunol.* **2020**, *145*, 37–53.
- 8. Hashemi, E.; Malarkannan, S. Tissue-Resident NK Cells: Development, Maturation, and Clinical Relevance. *Cancers* 2020, *12*, 1553. [CrossRef]
- 9. Li, H.; Hou, Y.; Zhang, S.; Zhou, Y.; Wang, D.; Tao, S.; Ni, F. CD49a regulates the function of human decidual natural killer cells. *Am. J. Reprod. Immunol.* **2019**, *81*, e13101. [CrossRef] [PubMed]
- 10. Kim, S.; Iizuka, K.; Kang, H.S.; Dokun, A.; French, A.R.; Greco, S.; Yokoyama, W.M. In vivo developmental stages in murine natural killer cell maturation. *Nat. Immunol.* **2002**, *3*, 523–528. [CrossRef] [PubMed]
- 11. Hayakawa, Y.; Smyth, M.J. CD27 dissects mature NK cells into two subsets with distinct responsiveness and migratory capacity. *J. Immunol.* 2006, 176, 1517–1524. [CrossRef]
- 12. Sojka, D.K.; Tian, Z.; Yokoyama, W.M. Tissue-resident natural killer cells and their potential diversity. *Semin. Immunol.* **2014**, *26*, 127–131. [CrossRef] [PubMed]
- 13. Sojka, D.K.; Yang, L.; Yokoyama, W.M. Uterine Natural Killer Cells. Front. Immunol. 2019, 10, 960. [CrossRef] [PubMed]
- 14. Bogdan, A.; Berta, G.; Szekeres-Bartho, J. PIBF positive uterine NK cells in the mouse decidua. *J. Reprod. Immunol.* **2017**, *119*, 38–43. [CrossRef] [PubMed]
- 15. Csabai, T.; Pallinger, E.; Kovacs, A.F.; Miko, E.; Bognar, Z.; Szekeres-Bartho, J. Altered Immune Response and Implantation Failure in Progesterone-Induced Blocking Factor-Deficient Mice. *Front. Immunol.* **2020**, *11*, 349. [CrossRef] [PubMed]
- Liang, Q.; Tong, L.; Xiang, L.; Shen, S.; Pan, C.; Liu, C.; Zhang, H. Correlations of the expression of γδ T cells and their costimulatory molecules TIGIT, PD-1, ICOS and BTLA with PR and PIBF in the peripheral blood and decidual tissues of women with unexplained recurrent spontaneous abortion. *Clin. Exp. Immunol.* 2021, 203, 55–65. [CrossRef]
- Lee, C.L.; Vijayan, M.; Wang, X.; Lam, K.K.W.; Koistinen, H.; Seppala, M.; Li, R.H.W.; Ng, E.H.Y.; Yeung, W.S.B.; Chiu, P.C.N. Glycodelin-A stimulates the conversion of human peripheral blood CD16-CD56bright NK cell to a decidual NK cell-like phenotype. *Hum. Reprod.* 2019, 34, 689–701. [CrossRef]
- 18. Dixit, A.; Karande, A.A. Glycodelin regulates the numbers and function of peripheral natural killer cells. *J. Reprod. Immunol.* **2020**, 137, 102625. [CrossRef]
- 19. Liu, Y.; Gao, S.; Zhao, Y.; Wang, H.; Pan, Q.; Shao, Q. Decidual Natural Killer Cells: A Good Nanny at the Maternal-Fetal Interface During Early Pregnancy. *Front. Immunol.* **2021**, *12*, 663660. [CrossRef]
- Feyaerts, D.; Kuret, T.; van Cranenbroek, B.; van der Zeeuw-Hingrez, S.; van der Heijden, O.W.H.; van der Meer, A.; Joosten, I.; van der Molen, R.G. Endometrial natural killer (NK) cells reveal a tissue-specific receptor repertoire. *Hum. Reprod* 2018, 33, 441–451. [CrossRef]
- 21. Mendes, J.; Areia, A.L.; Rodrigues-Santos, P.; Santos-Rosa, M.; Mota-Pinto, A. Innate Lymphoid Cells in Human Pregnancy. *Front. Immunol.* **2020**, *11*, 551707. [CrossRef]
- De Mendonça Vieira, R.; Meagher, A.; Crespo, Â.C.; Kshirsagar, S.K.; Iyer, V.; Norwitz, E.R.; Strominger, J.L.; Tilburgs, T. Human Term Pregnancy Decidual NK Cells Generate Distinct Cytotoxic Responses. J. Immunol. 2020, 204, 3149–3159. [CrossRef] [PubMed]
- 23. Huhn, O.; Ivarsson, M.A.; Gardner, L.; Hollinshead, M.; Stinchcombe, J.C.; Chen, P.; Shreeve, N.; Chazara, O.; Farrell, L.E.; Theorell, J.; et al. Distinctive phenotypes and functions of innate lymphoid cells in human decidua during early pregnancy. *Nat. Commun.* **2020**, *11*, 381. [CrossRef] [PubMed]
- 24. Guo, C.; Cai, P.; Jin, L.; Sha, Q.; Yu, Q.; Zhang, W.; Jiang, C.; Liu, Q.; Zong, D.; Li, K.; et al. Single-cell profiling of the human decidual immune microenvironment in patients with recurrent pregnancy loss. *Cell. Discov.* **2021**, *7*, 1. [CrossRef] [PubMed]
- 25. Li, T.; Cui, L.; Xu, X.; Zhang, H.; Jiang, Y.; Ren, L.; Yang, C.; Liu, X.; Hu, X. The Role of Tim-3 on dNK Cells Dysfunction During Abnormal Pregnancy With Toxoplasma gondii Infection. *Front. Cell. Infect. Microbiol.* **2021**, *11*, 587150. [CrossRef]
- Xu, X.; Zhang, J.; Zhan, S.; Li, Z.; Liu, X.; Zhang, H.; Jiang, Y.; Hu, X. TGF-β1 improving abnormal pregnancy outcomes induced by Toxoplasma gondii infection: Regulating NKG2D/DAP10 and killer subset of decidual NK cells. *Cell. Immunol.* 2017, 317, 9–17. [CrossRef]
- 27. Loverro, G.; Maiorano, E.; Napoli, A.; Selvaggi, L.; Marra, E.; Perlino, E. Transforming growth factor-beta 1 and insulin-like growth factor-1 expression in ovarian endometriotic cysts: A preliminary study. *Int. J. Mol. Med.* **2001**, *7*, 423–429.
- 28. Mizumoto, Y. Changes in NK activities and TGF- beta concentrations in the peritoneal cavity in endometriosis and their interaction related with infertility. *Nihon Sanka Fujinka Gakkai Zasshi* **1996**, *48*, 379–385. [PubMed]
- 29. Eriksson, M.; Meadows, S.K.; Wira, C.R.; Sentman, C.L. Unique phenotype of human uterine NK cells and their regulation by endogenous TGF-beta. *J. Leukoc. Biol.* 2004, *76*, 667–675. [CrossRef]
- 30. Chakraborty, D.; Rumi, M.A.; Soares, M.J. NK cells, hypoxia and trophoblast cell differentiation. *Cell. Cycle.* **2012**, *11*, 2427–2430. [CrossRef] [PubMed]

- Parodi, M.; Raggi, F.; Cangelosi, D.; Manzini, C.; Balsamo, M.; Blengio, F.; Eva, A.; Varesio, L.; Pietra, G.; Moretta, L.; et al. Hypoxia Modifies the Transcriptome of Human NK Cells, Modulates Their Immunoregulatory Profile, and Influences NK Cell Subset Migration. *Front. Immunol.* 2018, *9*, 2358. [CrossRef]
- Hawke, L.G.; Whitford, M.K.M.; Ormiston, M.L. The Production of Pro-angiogenic VEGF-A Isoforms by Hypoxic Human NK Cells Is Independent of Their TGF-β-Mediated Conversion to an ILC1-Like Phenotype. Front. Immunol. 2020, 25, 1903. [CrossRef]
- Thiruchelvam, U.; Wingfield, M.; O'Farrelly, C. Natural Killer Cells: Key Players in Endometriosis. Am. J. Reprod. Immunol. 2015, 74, 291–301. [CrossRef]
- 34. Shirasuna, K.; Iwata, H. Effect of aging on the female reproductive function. Contracept. Reprod. Med. 2017, 2, 23. [CrossRef]
- Chou, Y.C.; Chen, C.H.; Chen, M.J.; Chang, C.W.; Chen, P.H.; Yu, M.H.; Chen, Y.J.; Tsai, E.M.; Yang, P.S.; Lin, S.Y.; et al. Killer cell immunoglobulin-like receptors (KIR) and human leukocyte antigen-C (HLA-C) allorecognition patterns in women with endometriosis. *Sci. Rep.* 2020, *10*, 4897. [CrossRef]
- Lin, F.; Yang, C.; Feng, T.; Yang, S.; Zhou, R.; Li, H. The Maternal-Fetal Interface in Small-for-Gestational-Age Pregnancies Is Associated With a Reduced Quantity of Human Decidual NK Cells With Weaker Functional Ability. *Front. Cell. Dev. Biol.* 2020, 8, 633. [CrossRef] [PubMed]
- Papuchova, H.; Kshirsagar, S.; Xu, L.; Bougleux Gomes, H.A.; Li, Q.; Iyer, V.; Norwitz, E.R.; Strominger, J.L.; Tilburgs, T. Three types of HLA-G+ extravillous trophoblasts that have distinct immune regulatory properties. *Proc. Natl. Acad. Sci. USA*. 2020, 7, 15772–15777. [CrossRef] [PubMed]
- Yokomizo, R.; Fujiki, Y.; Kishigami, H.; Kishi, H.; Kiyono, T.; Nakayama, S.; Sago, H.; Okamoto, A.; Umezawa, A. Endometrial regeneration with endometrial epithelium: Homologous orchestration with endometrial stroma as a feeder. *Stem. Cell. Res. Ther.* 2021, 12, 130. [CrossRef] [PubMed]
- 39. Li, Y.; Li, R.; Ouyang, N.; Dai, K.; Yuan, P.; Zheng, L.; Wang, W. Investigating the impact of local inflammation on granulosa cells and follicular development in women with ovarian endometriosis. *Fertil. Steril.* **2019**, *112*, 882–891. [CrossRef]
- 40. Garmendia, J.V.; De Sanctis, J.B. Perspectives of new therapies for endometriosis. *Recent. Pat. Endocr. Metab. Immune Drug Discov.* **2012**, *6*, 218–223. [CrossRef]
- Zhang, Y.; Zhao, Y.; Si, W.; Yang, B.; Lin, M.; Zheng, J.; Cui, L. Increased Peripheral NKG2A-NKG2D+CD3-CD16+CD56dim NK Cell Subset Was Positively Correlated with Antiphospholipid Antibodies in Patients of Obstetric Antiphospholipid Syndrome. *Immunol. Invest.* 2020, 26, 1–14. [CrossRef]
- 42. Greenbaum, H.; Galper, B.L.; Decter, D.H.; Eisenberg, V.H. Endometriosis and autoimmunity: Can autoantibodies be used as a non-invasive early diagnostic tool? *Autoimmun. Rev.* **2021**, *20*, 102795. [CrossRef]
- 43. Kliemann, N.; Viallon, V.; Murphy, N.; Beeken, R.J.; Rothwell, J.A.; Rinaldi, S.; Assi, N.; van Roekel, E.H.; Schmidt, J.A.; Borch, K.B.; et al. Metabolic signatures of greater body size and their associations with risk of colorectal and endometrial cancers in the European Prospective Investigation into Cancer and Nutrition. *BMC Med.* 2021, 19, 101. [CrossRef] [PubMed]
- 44. Patel, M.V.; Shen, Z.; Rodriguez-Garcia, M.; Usherwood, E.J.; Tafe, L.J.; Wira, C.R. Endometrial Cancer Suppresses CD8+ T Cell-Mediated Cytotoxicity in Postmenopausal Women. *Front. Immunol.* **2021**, *12*, 657326. [CrossRef] [PubMed]
- 45. Liu, W.; Sun, L.; Zhang, J.; Song, W.; Li, M.; Wang, H. The landscape and prognostic value of immune characteristics in uterine corpus endometrial cancer. *Biosci. Rep.* 2021, *41*, BSR20202321. [CrossRef] [PubMed]
- Bähr, I.; Spielmann, J.; Quandt, D.; Kielstein, H. Obesity-Associated Alterations of Natural Killer Cells and Immunosurveillance of Cancer. *Front. Immunol.* 2020, 11, 245. [CrossRef] [PubMed]
- 47. Van der Molen, R.G.; Schutten, J.H.; van Cranenbroek, B.; ter Meer, M.; Donckers, J.; Scholten, R.R.; van der Heijden, O.W.; Spaanderman, M.E.; Joosten, I. Menstrual blood closely resembles the uterine immune micro-environment and is clearly distinct from peripheral blood. *Hum. Reprod.* **2014**, *29*, 303–314. [CrossRef]
- Tilburgs, T.; Scherjon, S.A.; Roelen, D.L.; Claas, F.H. Decidual CD8+CD28- T cells express CD103 but not perforin. *Hum. Immunol.* 2009, 70, 96–100. [CrossRef]
- 49. Kim, Y.; Shin, Y.; Kang, G.H. Prognostic significance of CD103+ immune cells in solid tumor: A systemic review and meta-analysis. *Sci. Rep.* **2019**, *9*, 3808. [CrossRef]
- Degos, C.; Heinemann, M.; Barrou, J.; Boucherit, N.; Lambaudie, E.; Savina, A.; Gorvel, L.; Olive, D. Endometrial Tumor Microenvironment Alters Human NK Cell Recruitment, and Resident NK Cell Phenotype and Function. *Front. Immunol.* 2019, 10, 877. [CrossRef]
- Marin, M.L.C.; Coelho, V.; Visentainer, J.E.L.; Alves, H.V.; Köhler, K.F.; Rached, M.R.; Abrão, M.S.; Kalil, J. Inhibitory KIR2DL2 Gene: Risk for Deep Endometriosis in Euro-descendants. *Reprod. Sci.* 2021, 28, 291–304. [CrossRef] [PubMed]
- 52. Bai, Y.; Liang, J.; Liu, W.; Wang, F.; Li, C. Possible roles of HLA-G regulating immune cells in pregnancy and endometrial diseases via KIR2DL4. *J. Reprod. Immunol.* 2020, 142, 103176. [CrossRef]
- 53. Xu, H. Expressions of natural cytotoxicity receptor, NKG2D and NKG2D ligands in endometriosis. *J. Reprod. Immunol.* 2019, 136, 102615. [CrossRef]
- 54. Freitag, N.; Pour, S.J.; Fehm, T.N.; Toth, B.; Markert, U.R.; Weber, M.; Togawa, R.; Kruessel, J.S.; Baston-Buest, D.M.; Bielfeld, A.P. Are uterine natural killer and plasma cells in infertility patients associated with endometriosis, repeated implantation failure, or recurrent pregnancy loss? *Arch. Gynecol. Obstet.* 2020, 302, 1487–1494. [CrossRef] [PubMed]
- Liu, Z.Q.; Lu, M.Y.; Liu, B. Circulating CD56⁺ NKG2D⁺ NK cells and postoperative fertility in ovarian endometrioma. *Sci. Rep.* 2020, 10, 18598. [CrossRef] [PubMed]

- Tincani, A.; Nalli, C.; Khizroeva, J.; Bitsadze, V.; Lojacono, A.; Andreoli, L.; Shoenfeld, Y.; Makatsariya, A. Autoimmune diseases and pregnancy. *Best Pract. Res. Clin. Endocrinol. Metab.* 2019, *33*, 101322. [CrossRef] [PubMed]
- Vomstein, K.; Feil, K.; Strobel, L.; Aulitzky, A.; Hofer-Tollinger, S.; Kuon, R.J.; Toth, B. Immunological Risk Factors in Recurrent Pregnancy Loss: Guidelines Versus Current State of the Art. J. Clin. Med. 2021, 10, 869. [CrossRef] [PubMed]
- Kwak-Kim, J.; Skariah, A.; Wu, L.; Salazar, D.; Sung, N.; Ota, K. Humoral and cellular autoimmunity in women with recurrent pregnancy losses and repeated implantation failures: A possible role of vitamin D. *Autoimmun. Rev.* 2016, 15, 943–947. [CrossRef] [PubMed]
- 59. Gridelet, V.; Perrier d'Hauterive, S.; Polese, B.; Foidart, J.M.; Nisolle, M.; Geenen, V. Human Chorionic Gonadotrophin: New Pleiotropic Functions for an "Old" Hormone During Pregnancy. *Front. Immunol.* **2020**, *11*, 343. [CrossRef]
- 60. Jiang, M.; Shen, N.; Zhou, H.; Wang, Y.; Lin, S.; Wu, J.; Di, W. The enrichment of neutrophil extracellular traps impair the placentas of systemic lupus erythematosus through accumulating decidual NK cells. *Sci. Rep.* **2021**, *11*, 6870. [CrossRef]
- 61. Wouters, K.; Kusters, Y.H.A.M.; Bijnen, M.; Wetzels, S.; Zhang, X.; Linssen, P.B.C.; Gaens, K.; Houben, A.J.H.M.; Joris, P.J.; Plat, J.; et al. NK cells in human visceral adipose tissue contribute to obesity-associated insulin resistance through low-grade inflammation. *Clin. Transl. Med.* **2020**, *10*, e192. [CrossRef]
- 62. Fernø, J.; Strand, K.; Mellgren, G.; Stiglund, N.; Björkström, N.K. Natural Killer Cells as Sensors of Adipose Tissue Stress. *Trends Endocrinol. Metab.* **2020**, *31*, 3–12. [CrossRef]
- 63. Spielmann, J.; Mattheis, L.; Jung, J.S.; Rauße, H.; Glaß, M.; Bähr, I.; Quandt, D.; Oswald, J.; Kielstein, H. Effects of obesity on NK cells in a mouse model of postmenopausal breast cancer. *Sci. Rep.* **2020**, *10*, 20606. [CrossRef]
- 64. Valero-Pacheco, N.; Beaulieu, A.M. Transcriptional Regulation of Mouse Tissue-Resident Natural Killer Cell Development. *Front. Immunol.* **2020**, *11*, 309. [CrossRef] [PubMed]
- 65. Guzik, T.J.; Skiba, D.S.; Touyz, R.M.; Harrison, D.G. The role of infiltrating immune cells in dysfunctional adipose tissue. *Cardiovasc Res.* **2017**, *113*, 1009–1023. [CrossRef]
- Braunschweig, A.; Poehlmann, T.G.; Busch, S.; Schleussner, E.; Markert, U.R. Signal transducer and activator of transcription 3 (STAT3) and Suppressor of Cytokine Signaling (SOCS3) balance controls cytotoxicity and IL-10 expression in decidual-like natural killer cell line NK-92. *Am. J. Reprod. Immunol.* 2011, *66*, 329–335. [CrossRef] [PubMed]
- 67. Tang, Y.; Sun, X.; Wang, Y.; Luan, H.; Zhang, R.; Hu, F.; Sun, X.; Li, X.; Guo, J. Role of IL-24 in NK cell activation and its clinical implication in systemic lupus erythematosus. *Clin. Rheumatol.* **2021**, *40*, 2707–2715. [CrossRef] [PubMed]
- Nörenberg, J.; Meggyes, M.; Jakso, P.; Miko, E.; Barakonyi, A. TIM-3 and TIM-1 Could Regulate Decidual γδTCR Bright T Cells during Murine Pregnancy. J. Immunol. Res. 2019, 2019, 3836942. [CrossRef]
- 69. Tohma, Y.A.; Musabak, U.; Gunakan, E.; Akilli, H.; Onalan, G.; Zeyneloglu, H.B. The Role of Analysis of NK Cell Subsets in Peripheral Blood and Uterine Lavage Samples in Evaluation of Patients with Recurrent Implantation Failure. *J. Gynecol. Obstet. Hum. Reprod.* **2020**, *49*, 101793. [CrossRef] [PubMed]
- Sureshchandra, S.; Marshall, N.E.; Messaoudi, I. Impact of pregravid obesity on maternal and fetal immunity: Fertile grounds for reprogramming. J. Leukoc. Biol. 2019, 106, 1035–1050. [CrossRef]
- 71. Castellana, B.; Perdu, S.; Kim, Y.; Chan, K.; Atif, J.; Marziali, M.; Beristain, A.G. Maternal obesity alters uterine NK activity through a functional KIR2DL1/S1 imbalance. *Immunol. Cell. Biol.* **2018**, *96*, 805–819. [CrossRef]
- 72. St-Germain, L.E.; Castellana, B.; Baltayeva, J.; Beristain, A.G. Maternal Obesity and the Uterine Immune Cell Landscape: The Shaping Role of Inflammation. *Int. J. Mol. Sci.* 2020, *21*, 3776. [CrossRef] [PubMed]
- Barra, N.G.; Fan, I.Y.; Gillen, J.B.; Chew, M.; Marcinko, K.; Steinberg, G.R.; Gibala, M.J.; Ashkar, A.A. High Intensity Interval Training Increases Natural Killer Cell Number and Function in Obese Breast Cancer-challenged Mice and Obese Women. *J Cancer Prev.* 2017, 22, 260–266. [CrossRef]
- 74. Sappenfield, E.; Jamieson, D.J.; Kourtis, A.P. Pregnancy and susceptibility to infectious diseases. *Infect. Dis. Obstet. Gynecol.* 2013, 2013, 752852. [CrossRef]
- Cornish, E.F.; Filipovic, I.; Åsenius, F.; Williams, D.J.; McDonnell, T. Innate Immune Responses to Acute Viral Infection During Pregnancy. *Front. Immunol.* 2020, 11, 572567. [CrossRef] [PubMed]
- 76. Parker, E.L.; Silverstein, R.B.; Verma, S.; Mysorekar, I.U. Viral-Immune Cell Interactions at the Maternal-Fetal Interface in Human Pregnancy. *Front. Immunol.* 2020, *11*, 522047. [CrossRef] [PubMed]
- 77. Lu-Culligan, A.; Chavan, A.R.; Vijayakumar, P.; Irshaid, L.; Courchaine, E.M.; Milano, M.; Tang, Z.; Pope, S.D.; Song, E.; Vogels, C.B.F.; et al. Maternal respiratory SARS-CoV-2 infection in pregnancy is associated with a robust inflammatory response at the maternal-fetal interface. *Medicine* 2021, 2, 591–610. [CrossRef]
- 78. Barber, E.M.; Pollard, J.W. The uterine NK cell population requires IL-15, but these cells are not required for pregnancy nor the resolution of a Listeria monocytogenes infection. *J. Immunol.* **2003**, 171, 37–46. [CrossRef]
- Clayton, R.D.; Duffy, S.R.; Wilkinson, N.; Garry, R.; Jackson, A.M. Increase in peripheral blood mononuclear cell (PBMC)—And CD56+ cell-mediated killing of endometrial stromal cells by mycobacteria; a possible role in endometriosis immunotherapy? *Hum. Reprod.* 2004, 19, 1886–1893. [CrossRef] [PubMed]
- Omosun, Y.O.; Blackstock, A.J.Ñ.; Gatei, W.; Hightower, A.; van Eijk, A.M.; Ayisi, J.; Otieno, J.; Lal, R.B.; Steketee, R.; Nahlen, B.; et al. Differential association of gene content polymorphisms of killer cell immunoglobulin-like receptors with placental malaria in HIV- and HIV+ mothers. *PLoS ONE* 2012, 7, e38617. [CrossRef]

- Omosun, Y.O.; Blackstock, A.J.; Williamson, J.; van Eijk, A.M.; Ayisi, J.; Otieno, J.; Lal, R.B.; Ter Kuile, F.O.; Slutsker, L.; Shi, Y.P. Association of maternal KIR gene content polymorphisms with reduction in perinatal transmission of HIV-1. *PLoS ONE* 2018, 13, e0191733. [CrossRef] [PubMed]
- Damelang, T.; Aitken, E.H.; Hasang, W.; Lopez, E.; Killian, M.; Unger, H.W.; Salanti, A.; Shub, A.; McCarthy, E.; Kedzierska, K.; et al. Antibody-mediated activation of natural killer cells in malaria exposed pregnant women. *Sci. Rep.* 2021, *11*, 4130. [CrossRef] [PubMed]
- 83. Shmeleva, E.V.; Colucci, F. Maternal natural killer cells at the intersection between reproduction and mucosal immunity. *Mucosal. Immunol.* **2021**, *26*, 1–15.
- 84. Colucci, F.; Traherne, J. Killer-cell immunoglobulin-like receptors on the cusp of modern immunogenetics. *Immunology* **2017**, 152, 556–561. [CrossRef] [PubMed]
- Huhn, O.; Chazara, O.; Ivarsson, M.A.; Retière, C.; Venkatesan, T.C.; Norman, P.J.; Hilton, H.G.; Jayaraman, J.; Traherne, J.A.; Trowsdale, J.; et al. High-Resolution Genetic and Phenotypic Analysis of KIR2DL1 Alleles and Their Association with Pre-Eclampsia. J. Immunol. 2018, 201, 2593–2601. [CrossRef] [PubMed]
- van 't Hof, L.J.; Schotvanger, N.; Haasnoot, G.W.; van der Keur, C.; Roelen, D.L.; Lashley, L.E.E.L.O.; Claas, F.H.J.; Eikmans, M.; van der Hoorn, M.P. Maternal-Fetal HLA Compatibility in Uncomplicated and Preeclamptic Naturally Conceived Pregnancies. *Front. Immunol.* 2021, 12, 673131. [CrossRef] [PubMed]
- 87. Han, M.; Jiang, Y.; Lao, K.; Xu, X.; Zhan, S.; Wang, Y.; Hu, X. sHLA-G involved in the apoptosis of decidual natural killer cells following Toxoplasma gondii infection. *Inflammation* **2014**, *37*, 1718–1727. [CrossRef] [PubMed]
- Tomac, J.; Mazor, M.; Lisnić, B.; Golemac, M.; Kveštak, D.; Bralić, M.; Bilić Zulle, L.; Brinkmann, M.M.; Dölken, L.; Reinert, L.S.; et al. Viral infection of the ovaries compromises pregnancy and reveals innate immune mechanisms protecting fertility. *Immunity* 2021. [CrossRef]
- Kujur, W.; Murillo, O.; Adduri, R.S.R.; Vankayalapati, R.; Konduru, N.V.; Mulik, S. Memory like NK cells display stem cell like properties after Zika virus infection. *PLoS Pathog.* 2020, 16, e1009132. [CrossRef]
- 90. Glasner, A.; Oiknine-Djian, E.; Weisblum, Y.; Diab, M.; Panet, A.; Wolf, D.G.; Mandelboim, O. Zika Virus Escapes NK Cell Detection by Upregulating Major Histocompatibility Complex Class I Molecules. J. Virol. 2017, 91, e00785-17. [CrossRef]
- 91. Schust, D.J.; Hill, A.B.; Ploegh, H.L. Herpes simplex virus blocks intracellular transport of HLA-G in placentally derived human cells. *J. Immunol.* **1996**, *157*, 3375–3380.
- 92. Li, L.; Wang, L.; Huang, C.; Diao, L.; Zhang, Y.; Zhang, X.; Xu, J.; Zeng, Y. Chronic hepatitis B infection alters peripheral immune response in women with reproductive failure. *Am. J. Reprod. Immunol.* **2019**, *81*, e13083. [CrossRef] [PubMed]
- 93. Corado, J.; Toro, F.; Rivera, H.; Bianco, N.E.; Deibis, L.; De Sanctis, J.B. Impairment of natural killer (NK) cytotoxic activity in hepatitis C virus (HCV) infection. *Clin. Exp. Immunol.* **1997**, *109*, 451–457. [CrossRef] [PubMed]
- Shabrish, S.; Karnik, N.; Gupta, V.; Bhate, P.; Madkaikar, M. Impaired NK cell activation during acute dengue virus infection: A contributing factor to disease severity. *Heliyon* 2020, *6*, e04320. [CrossRef] [PubMed]
- 95. Wagstaffe, H.R.; Susannini, G.; Thiébaut, R.; Richert, L.; Lévy, Y.; Bockstal, V.; Stoop, J.N.; Luhn, K.; Douoguih, M.; Riley, E.M.; et al. Durable natural killer cell responses after heterologous two-dose Ebola vaccination. *NPJ Vaccines*. **2021**, *6*, 19. [CrossRef] [PubMed]
- 96. Björkström, N.K.; Strunz, B.; Ljunggren, H.G. Natural killer cells in antiviral immunity. Nat. Rev. Immunol. 2021, 11, 1–12.
- 97. Piersma, S.J.; Brizić, I. Natural Killer cell effector functions in antiviral defense. FEBS J. 2021. [CrossRef]
- Ścieżyńska, A.; Komorowski, M.; Soszyńska, M.; Malejczyk, J. NK Cells as Potential Targets for Immunotherapy in Endometriosis. J. Clin. Med. 2019, 8, 1468. [CrossRef] [PubMed]
- Dizaji Asl, K.; Velaei, K.; Rafat, A.; Tayefi Nasrabadi, H.; Movassaghpour, A.A.; Mahdavi, M.; Nozad Charoudeh, H. The role of KIR positive NK cells in diseases and its importance in clinical intervention. *Int. Immunopharmacol.* 2021, 92, 107361. [CrossRef] [PubMed]
- Guerrero, B.; Hassouneh, F.; Delgado, E.; Casado, J.G.; Tarazona, R. Natural killer cells in recurrent miscarriage: An overview. J. Reprod. Immunol. 2020, 142, 103209. [CrossRef] [PubMed]
- 101. Zhang, H.; Qi, J.; Wang, Y.; Sun, J.; Li, Z.; Sui, L.; Fan, J.; Liu, C.; Shang, Y.; Kong, L.; et al. Progesterone Regulates Glucose Metabolism Through Glucose Transporter 1 to Promote Endometrial Receptivity. *Front. Physiol.* **2020**, *11*, 543148. [CrossRef]
- 102. Ata, B.; Telek, S.B. Assisted reproductive technology for women with endometriosis, a clinically oriented review. *Curr. Opin. Obstet. Gynecol.* **2021**, *33*, 225–231. [CrossRef] [PubMed]
- 103. He, J.; Xu, Y.; Yi, M.; Gu, C.; Zhu, Y.; Hu, G. Involvement of natural killer cells in the pathogenesis of endometriosis in patients with pelvic pain. *J. Int. Med. Res.* 2020, *48.* [CrossRef] [PubMed]
- 104. Busnelli, A.; Somigliana, E.; Cirillo, F.; Baggiani, A.; Levi-Setti, P.E. Efficacy of therapies and interventions for repeated embryo implantation failure: A systematic review and meta-analysis. *Sci. Rep.* **2021**, *11*, 1747. [CrossRef]
- 105. Szekeres-Bartho, J.; Schindler, A.E. Progestogens and immunology. *Best Pract. Res Clin. Obstet. Gynaecol.* 2019, 60, 17–23. [CrossRef]
- 106. Miko, E.; Halasz, M.; Jericevic-Mulac, B.; Wicherek, L.; Arck, P.; Arató, G.; Skret Magierlo, J.; Rukavina, D.; Szekeres-Bartho, J. Progesterone-induced blocking factor, (PIBF) and trophoblast invasiveness. J. Reprod. Immunol. 2011, 90, 50–57. [CrossRef]

- 107. Wilsher, S.; Newcombe, J.R.; Allen, W.R.T. The immunolocalisation of Galectin-1 and Progesterone-Induced Blocking Factor (PIBF) in equine trophoblast: Possible roles in trophoblast invasion and the immunological protection of pregnancy. *Placenta* 2019, 85, 32–39. [CrossRef]
- 108. Murata, H.; Taaka, S.; Okada, H. Immune Tolerance of the Human Decidua. J. Clin. Med. 2021, 10, 351. [CrossRef] [PubMed]
- Wang, F.; Zhu, H.; Li, B.; Liu, M.; Liu, D.; Deng, M.; Wang, Y.; Xia, X.; Jiang, Q.; Chen, D. Effects of human chorionic gonadotropin, estradiol, and progesterone on interleukin-18 expression in human decidual tissues. *Gynecol. Endocrinol.* 2017, 33, 265–269. [CrossRef]
- 110. Murata, H.; Tanaka, S.; Tsuzuki-Nakao, T.; Kido, T.; Kakita-Kobayashi, M.; Kida, N.; Hisamatsu, Y.; Tsubokura, H.; Hashimoto, Y.; Kitada, M.; et al. The transcription factor HAND2 up-regulates transcription of the IL15 gene in human endometrial stromal cells. *J. Biol. Chem.* 2020, 295, 9596–9605. [CrossRef]
- 111. Thomsen, L.H.; Kesmodel, U.S.; Erb, K.; Bungum, L.; Pedersen, D.; Hauge, B.; Elbæk, H.O.; Povlsen, B.B.; Andersen, C.Y.; Humaidan, P. The impact of luteal serum progesterone levels on live birth rates-a prospective study of 602 IVF/ICSI cycles. *Hum. Reprod.* 2018, 33, 1506–1516. [CrossRef]
- 112. Martínez, M.C.; Rodríguez-Varela, C.; Demur, E.L. New concepts and difficulties with progesterone supplementation in the luteal phase. *Curr. Opin.Obstet. Gynecol.* 2021, 33, 196–201. [CrossRef] [PubMed]
- 113. Cho, K.; Kook, H.; Kang, S.; Lee, J. Study of immune-tolerised cell lines and extracellular vesicles inductive environment promoting continuous expression and secretion of HLA-G from semiallograft immune tolerance during pregnancy. *J. Extracell. Vesicles.* **2020**, *9*, 1795364. [CrossRef]
- 114. Johann, S.; Zoller, C.; Haas, S.; Blümel, G.; Lipp, M.; Förster, R. Sulfated polysaccharide anticoagulants suppress natural killer cell activity in vitro. *Thromb. Haemost.* **1995**, *74*, 998–1002. [CrossRef] [PubMed]
- 115. Tamaru, S.; Kajihara, T.; Mizuno, Y.; Takano, N.; Tochigi, H.; Sato, T.; Ishihara, O. Heparin prevents oxidative stress-induced apoptosis in human decidualised endometrial stromal cells. *Med. Mol. Morphol.* **2019**, *52*, 209–216. [CrossRef]
- 116. Dias, A.T.B.; Modesto, T.B.; Oliveira, S.A. Effectiveness of the use of Low Molecular Heparin in patients with repetition abortion history: Systematic review and meta-analysis. *JBRA Assist. Reprod.* **2021**, *25*, 10–27.
- 117. Bruno, V.; Svensson-Arvelund, J.; Rubér, M.; Berg, G.; Piccione, E.; Jenmalm, M.C.; Ernerudh, J. Effects of low molecular weight heparin on the polarisation and cytokine profile of macrophages and T helper cells in vitro. *Sci. Rep.* **2018**, *8*, 4166. [CrossRef]
- 118. Rossi, G.R.; Gonçalves, J.P.; McCulloch, T.; Delconte, R.B.; Hennessy, R.J.; Huntington, N.D.; Trindade, E.S.; Souza-Fonseca-Guimaraes, F. The Antitumor Effect of Heparin is not Mediated by Direct NK Cell Activation. J. Clin. Med. 2020, 9, 2666. [CrossRef]
- 119. Lee, E.E.; Jun, J.K.; Lee, E.B. Management of Women with Antiphospholipid Antibodies or Antiphospholipid Syndrome during Pregnancy. J. Korean Med. Sci. 2021, 36, e24. [CrossRef]
- 120. Shimba, A.; Ikuta, K. Control of immunity by glucocorticoids in health and disease. *Semin. Immunopathol.* **2020**, 42, 669–680. [CrossRef]
- 121. Thum, M.Y.; Bhaskaran, S.; Abdalla, H.I.; Ford, B.; Sumar, N.; Bansal, A. Prednisolone suppresses NK cell cytotoxicity in vitro in women with a history of infertility and elevated NK cell cytotoxicity. *Am. J. Reprod. Immunol.* 2008, 59, 259–265. [CrossRef] [PubMed]
- 122. Gomaa, M.F.; Elkholy, A.G.; El-Said, M.M.; Abdel-Salam, N.E. Combined oral prednisolone and heparin versus heparin: The effect on peripheral NK cells and clinical outcome in patients with unexplained recurrent miscarriage. A double-blind placebo randomised controlled trial. *Arch. Gynecol. Obstet.* **2014**, *290*, 757–762. [CrossRef]
- 123. Wdowiak, A.; Raczkiewicz, D.; Janczyk, P.; Bojar, I.; Makara-Studzińska, M.; Wdowiak-Filip, A. Interactions of Cortisol and Prolactin with Other Selected Menstrual Cycle Hormones Affecting the Chances of Conception in Infertile Women. *Int. J. Environ. Res. Public Health* **2020**, *17*, 7537. [CrossRef]
- 124. Li, T.; Chen, Y.; Lai, Y.; He, G.; He, G. Expression and significance of PD-1 and PD-L1 in patients with recurrent spontaneous abortion: A protocol for systematic review and meta-analysis. *Medicine* **2021**, *100*, e25444. [CrossRef] [PubMed]
- 125. Wu, L.; Lv, C.; Su, Y.; Li, C.; Zhang, H.; Zhao, X.; Li, M. Expression of programmed death-1 (PD-1) and its ligand PD-L1 is upregulated in endometriosis and promoted by 17beta-estradiol. *Gynecol. Endocrinol.* **2019**, *35*, 251–256. [CrossRef] [PubMed]
- 126. Aston, W.J.; Hope, D.E.; Cook, A.M.; Boon, L.; Dick, I.; Nowak, A.K.; Lake, R.A.; Lesterhuis, W.J. Dexamethasone differentially depletes tumour and peripheral blood lymphocytes and can impact the efficacy of chemotherapy/checkpoint blockade combination treatment. *Oncoimmunology* 2019, *8*, e1641390. [CrossRef]
- 127. Ahmadi, M.; Ghaebi, M.; Abdolmohammadi-Vahid, S.; Abbaspour-Aghdam, S.; Hamdi, K.; Abdollahi-Fard, S.; Danaii, S.; Mosapour, P.; Koushaeian, L.; Dolati, S.; et al. NK cell frequency and cytotoxicity in correlation to pregnancy outcome and response to IVIG therapy among women with recurrent pregnancy loss. *J. Cell. Physiol.* **2019**, 234, 9428–9437. [CrossRef]
- 128. Jafarzadeh, S.; Ahmadi, M.; Dolati, S.; Aghebati-Maleki, L.; Eghbal-Fard, S.; Kamrani, A.; Behrad, B.; Roshangar, L.; Jadidi-Niaragh, F.; Yousefi, B.; et al. Intravenous immunoglobulin G treatment increases live birth rate in women with recurrent miscarriage and modulates regulatory and exhausted regulatory T cells frequency and function. J. Cell. Biochem. 2019, 120, 5424–5434. [CrossRef]
- 129. Christiansen, O.B.; Kolte, A.M.; Krog, M.C.; Nielsen, H.S.; Egerup, P. Treatment with intravenous immunoglobulin in patients with recurrent pregnancy loss: An update. *J. Reprod. Immunol.* **2019**, *133*, 37–42. [CrossRef]

- 130. Abdolmohammadi-Vahid, S.; Pashazadeh, F.; Pourmoghaddam, Z.; Aghebati-Maleki, L.; Abdollahi-Fard, S.; Yousefi, M. The effectiveness of IVIG therapy in pregnancy and live birth rate of women with recurrent implantation failure (RIF): A systematic review and meta-analysis. *J. Reprod. Immunol.* **2019**, *134–135*, 28–33. [CrossRef]
- 131. Roussev, R.G.; Ng, S.C.; Coulam, C.B. Natural killer cell functional activity suppression by intravenous immunoglobulin, intralipid and soluble human leukocyte antigen-G. *Am. J. Reprod. Immunol.* **2007**, *57*, 262–269. [CrossRef]
- 132. Meng, L.; Lin, J.; Chen, L.; Wang, Z.; Liu, M.; Liu, Y.; Chen, X.; Zhu, L.; Chen, H.; Zhang, J. Effectiveness and potential mechanisms of intralipid in treating unexplained recurrent spontaneous abortion. *Arch. Gynecol. Obstet.* **2016**, 294, 29–39. [CrossRef]
- 133. Lédée, N.; Vasseur, C.; Petitbarat, M.; Chevrier, L.; Vezmar, K.; Dray, G.; Chenière, S.; Lobersztajn, A.; Vitoux, D.; Cassuto, G.N.; et al. Intralipid®may represent a new hope for patients with reproductive failures and simultaneously an over-immune endometrial activation. *J. Reprod. Immunol.* **2018**, *130*, 18–22. [CrossRef]
- 134. Roussev, R.G.; Acacio, B.; Ng, S.C.; Coulam, C.B. Duration of intralipid's suppressive effect on NK cell's functional activity. *Am. J. Reprod. Immunol.* 2008, 60, 258–263. [CrossRef]
- 135. Canella, P.R.B.C.; Barini, R.; Carvalho, P.O.; Razolli, D.S. Lipid emulsion therapy in women with recurrent pregnancy loss and repeated implantation failure: The role of abnormal natural killer cell activity. J. Cell. Mol. Med. 2021, 25, 2290–2296. [CrossRef] [PubMed]
- 136. Kolanska, K.; Alijotas-Reig, J.; Cohen, J.; Cheloufi, M.; Selleret, L.; d'Argent, E.; Kayem, G.; Valverde, E.E.; Fain, O.; Bornes, M.; et al. Endometriosis with infertility: A comprehensive review on the role of immune deregulation and immunomodulation therapy. Am. J. Reprod. Immunol. 2021, 85, e13384. [CrossRef]
- 137. De Sanctis, J.B.; Blanca, I.; Bianco, N.E. Expression of different lipoprotein receptors in natural killer cells and their effect on natural killer proliferative and cytotoxic activity. *Immunology* **1995**, *86*, 399–407. [PubMed]
- De Sanctis, J.B.; Dumut, D.C.; Radzioch, D.; Hajdúch, M. Functionally Relevant Differences in Plasma Fatty Acid Composition and Expression of Cytotoxic and Inhibitory NK Cell Receptors between Healthy Young and Healthy Elder Adults. *Nutrients* 2020, 12, 3641. [CrossRef] [PubMed]
- 139. Evans, K.N.; Nguyen, L.; Chan, J.; Innes, B.A.; Bulmer, J.N.; Kilby, M.D.; Hewison, M. Effects of 25-hydroxyvitamin D3 and 1,25-dihydroxyvitamin D3 on cytokine production by human decidual cells. *Biol. Reprod.* **2006**, *75*, 816–822. [CrossRef]
- Tamblyn, J.A.; Jeffery, L.E.; Susarla, R.; Lissauer, D.M.; Coort, S.L.; Garcia, A.M.; Knoblich, K.; Fletcher, A.L.; Bulmer, J.N.; Kilby, M.D.; et al. Transcriptomic analysis of vitamin D responses in uterine and peripheral NK cells. *Reproduction* 2019, 158, 211–221. [CrossRef]
- Ota, K.; Dambaeva, S.; Kim, M.W.; Han, A.R.; Fukui, A.; Gilman-Sachs, A.; Beaman, K.; Kwak-Kim, J. 1,25-dihydroxy-vitamin D3 regulates NK-cell cytotoxicity, cytokine secretion, and degranulation in women with recurrent pregnancy losses. *Eur. J. Immunol.* 2015, 45, 3188–3199. [CrossRef]
- 142. Chen, X.; Yin, B.; Lian, R.C.; Zhang, T.; Zhang, H.Z.; Diao, L.H.; Li, Y.Y.; Huang, C.Y.; Liang, D.S.; Zeng, Y. Modulatory effects of vitamin D on peripheral cellular immunity in patients with recurrent miscarriage. *Am. J. Reprod. Immunol.* 2016, 76, 432–438. [CrossRef]
- 143. Ota, K.; Takahashi, T.; Han, A.; Damvaeba, S.; Mizunuma, H.; Kwak-Kim, J. Effects of MTHFR C677T polymorphism on vitamin D, homocysteine and natural killer cell cytotoxicity in women with recurrent pregnancy losses. *Hum. Reprod.* 2020, 35, 1276–1287. [CrossRef] [PubMed]
- 144. Zhao, H.; Wei, X.; Yang, X. A novel update on vitamin D in recurrent pregnancy loss. Mol. Med. Rep. 2021, 23, 382. [CrossRef]
- 145. Zhang, W.B.; Cheng, M.J.; Huang, Y.T.; Jiang, W.; Cong, Q.; Zheng, Y.F.; Xu, C.J. A study in vitro on differentiation of bone marrow mesenchymal stem cells into endometrial epithelial cells in mice. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 2012, 160, 185–190. [CrossRef]
- 146. Tempest, N.; Maclean, A.; Hapangama, D.K. Endometrial Stem Cell Markers: Current Concepts and Unresolved Questions. Int. J. Mol. Sci. 2018, 19, 3240. [CrossRef]
- 147. Rezaei Kahmini, F.; Shahgaldi, S.; Moazzeni, S.M. Mesenchymal stem cells alter the frequency and cytokine profile of natural killer cells in abortion-prone mice. *J. Cell. Physiol.* **2020**, 235, 7214–7223. [CrossRef] [PubMed]
- 148. Esfandyari, S.; Elkafas, H.; Chugh, R.M.; Park, H.S.; Navarro, A.; Al-Hendy, A. Exosomes as Biomarkers for Female Reproductive Diseases Diagnosis and Therapy. *Int. J. Mol. Sci.* **2021**, *22*, 2165. [CrossRef] [PubMed]
- 149. Wu, J.; Huang, H.; Huang, W.; Wang, L.; Xia, X.; Fang, X. Analysis of exosomal lncRNA, miRNA and mRNA expression profiles and ceRNA network construction in endometriosis. *Epigenomics* **2020**, *12*, 1193–1213. [CrossRef] [PubMed]
- 150. Wu, J.; Fang, X.; Huang, H.; Huang, W.; Wang, L.; Xia, X. Construction and topological analysis of an endometriosis-related exosomal circRNA-miRNA-mRNA regulatory network. *Aging* **2021**, *26*, 13.
- 151. Xu, P.; Ma, Y.; Wu, H.; Wang, Y.L. Placenta-Derived MicroRNAs in the Pathophysiology of Human Pregnancy. *Front. Cell. Dev. Biol.* **2021**, *9*, 646326. [CrossRef]
- 152. Kharazi, U.; Badalzadeh, R. A review on the stem cell therapy and an introduction to exosomes as a new tool in reproductive medicine. *Reprod. Biol.* 2020, 20, 447–459. [CrossRef]
- 153. Rodriguez-Caro, H.; Dragovic, R.; Shen, M.; Dombi, E.; Mounce, G.; Field, K.; Meadows, J.; Turner, K.; Lunn, D.; Child, T.; et al. In vitro decidualisation of human endometrial stromal cells is enhanced by seminal fluid extracellular vesicles. *J. Extracell. Vesicles* 2019, *8*, 1565262. [CrossRef] [PubMed]

- 154. Qi, Q.; Liu, X.; Zhang, Q.; Guo, S.W. Platelets induce increased estrogen production through NF-κB and TGF-β1 signaling pathways in endometriotic stromal cells. *Sci. Rep.* **2020**, *10*, 1281. [CrossRef]
- 155. Pourakbari, R.; Ahmadi, H.; Yousefi, M.; Aghebati-Maleki, L. Cell therapy in female infertility-related diseases: Emphasis on recurrent miscarriage and repeated implantation failure. *Life Sci.* 2020, 258, 118181. [CrossRef] [PubMed]
- 156. Strunz, B.; Bister, J.; Jönsson, H.; Filipovic, I.E.; Sleiers, N.; Dumitrescu, B.; Brännström, M.; Lentini, A.; Reinius, B.; Brnillet, M.; et al. Continuous human uterine NK cell differentiation in response to endometrial regeneration and pregnancy. *Sci. Immunol.* 2021, *6*, eabb7800. [CrossRef]
- 157. Aneman, I.; Pienaar, D.; Suvakov, S.; Simic, T.P.; Garovic, V.D.; McClements, L. Mechanisms of Key Innate Immune Cells in Earlyand Late-Onset Preeclampsia. *Front. Immunol.* 2020, *11*, 1864. [CrossRef]
- 158. Sun, R.; Xiong, Y.; Liu, H.; Gao, C.; Su, L.; Weng, J.; Yuan, X.; Zhang, D.; Feng, J. Tumor-associated neutrophils suppress antitumor immunity of NK cells through the PD-L1/PD-1 axis. *Transl. Oncol.* **2020**, *13*, 100825. [CrossRef] [PubMed]
- Okoye, I.S.; Xu, L.; Walker, J.; Elahi, S. The glucocorticoids prednisone and dexamethasone differentially modulate T cell function in response to anti-PD-1 and anti-CTLA-4 immune checkpoint blockade. *Cancer Immunol. Immunother.* 2020, 69, 1423–1436. [CrossRef] [PubMed]
- 160. Socha, M.W.; Malinowski, B.; Puk, O.; Wartęga, M.; Stankiewicz, M.; Kazdepka-Ziemińska, A.; Wiciński, M. The Role of NF-κB in Uterine Spiral Arteries Remodeling, Insight into the Cornerstone of Preeclampsia. *Int. J. Mol. Sci.* 2021, 22, 704. [CrossRef]
- Yang, J.H.; Chen, C.D.; Wu, M.Y.; Chao, K.H.; Yang, Y.S.; Ho, H.N. Hormone replacement therapy reverses the decrease in natural killer cytotoxicity but does not reverse the decreases in the T-cell subpopulation or interferon-gamma production in postmenopausal women. *Fertil.* 2000, 74, 261–267. [CrossRef]
- Waiyaput, W.; Wattanakamolchai, K.; Tingthanatikul, Y.; Lertvikool, S.; Tantanavipas, S.; Dittharot, K.; Sroyraya, M.; Sophonsritsuk, A. Effect of combined contraceptive pill on immune cell of ovarian endometriotic tissue. *J. Ovarian. Res.* 2021, 14, 66. [CrossRef]
- 163. Lai, Z.Z.; Yang, H.L.; Shi, J.W.; Shen, H.H.; Wang, Y.; Chang, K.K.; Zhang, T.; Ye, J.F.; Sun, J.S.; Qiu, X.M.; et al. Protopanaxadiol improves endometriosis associated infertility and miscarriage in sex hormones receptors-dependent and independent manners. *Int. J. Biol. Sci.* 2021, *17*, 1878–1894. [CrossRef] [PubMed]
- 164. Fu, Q.; Sun, Y.; Tao, Y.; Piao, H.; Wang, X.; Luan, X.; Du, M.; Li, D. Involvement of the JAK-STAT pathway in collagen regulation of decidual NK cells. *Am. J. Reprod. Immunol.* **2017**, *78*, 119. [CrossRef] [PubMed]
- 165. Vian, L.; Le, M.T.; Gazaniga, N.; Kieltyka, J.; Liu, C.; Pietropaolo, G.; Dell'Orso, S.; Brooks, S.R.; Furumoto, Y.; Thomas, C.J.; et al. JAK Inhibition Differentially Affects NK Cell and ILC1 Homeostasis. *Front. Immunol.* **2019**, *10*, 2972. [CrossRef] [PubMed]
- 166. Zhang, J.Y.; Wu, P.; Chen, D.; Ning, F.; Lu, Q.; Qiu, X.; Hewison, M.; Tamblyn, J.A.; Kilby, M.D.; Lash, G.E. Vitamin D Promotes Trophoblast Cell Induced Separation of Vascular Smooth Muscle Cells in Vascular Remodeling via Induction of G-CSF. *Front. Cell. Dev. Biol.* 2020, *8*, 601043. [CrossRef] [PubMed]